



Europäisches Patentamt
European Patent Office
Office européen des brevets



Publication number : **0 606 734 A1**

12

EUROPEAN PATENT APPLICATION

21 Application number : 93309950.9

22 Date of filing : 10.12.93

51 Int. Cl.⁵ : **C12N 15/12, C07K 15/06,
C12N 5/10, C12P 21/00,
G01N 33/50, C12P 21/08,
C12Q 1/68**

30 Priority : 11.12.92 US 987953

43 Date of publication of application :
20.07.94 Bulletin 94/29

84 Designated Contracting States :
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC
NL PT SE**

71 Applicant : **Foldes, Robert L.**
127 Chelmsford Avenue
Willowdale, Ontario M2R 2W6 (CA)

71 Applicant : **Kamboj, Rajender**
2869 Arvida Circle
Mississauga, Ontario L5N 1R4 (CA)

72 Inventor : **Foldes, Robert L.**
127 Chelmsford Avenue
Willowdale, Ontario M2R 2W6 (CA)
Inventor : **Kamboj, Rajender**
2869 Arvida Circle
Mississauga, Ontario L5N 1R4 (CA)

74 Representative : **Mercer, Christopher Paul**
Carpmaels & Ransford
43, Bloomsbury Square
London WC1A 2RA (GB)

54 Human CNS receptors of the NMDA-R1 family.

57 Neurotransmission by excitatory amino acids (EAAs) such as glutamate is mediated via membrane-bound surface receptors. DNA coding for EAA receptors of one family of human NMDA-binding type receptors has now been isolated and receptor proteins characterized. Herein described are recombinant cell lines which produce the EAA receptor as a heterologous membrane-bound product. Also described are related aspects of the invention, which are of commercial significance. Included is use of the cell lines as a tool for discovery of compounds which modulate EAA receptor stimulation.

EP 0 606 734 A1

Field of the Invention

This invention is concerned with applications of recombinant DNA technology in the field of neurobiology. More particularly, the invention relates to the cloning and expression of DNA coding for excitatory amino acid (EAA) receptors, especially human EAA receptors.

Background to the Invention

In the mammalian central nervous system (CNS), the transmission of nerve impulses is controlled by the interaction between a neurotransmitter substance released by the "sending" neuron which then binds to a surface receptor on the "receiving" neuron, to cause excitation thereof. L-glutamate is the most abundant neurotransmitter in the CNS, and mediates the major excitatory pathway in vertebrates. Glutamate is therefore referred to as an excitatory amino acid (EAA) and the receptors which respond to it are variously referred to as glutamate receptors, or more commonly as EAA receptors.

Using tissues isolated from mammalian brain, and various synthetic EAA receptor agonists, knowledge of EAA receptor pharmacology has been refined somewhat. Members of the EAA receptor family can be grouped into three main types based on differential binding to such agonists. One type of EAA receptor, which in addition to glutamate also binds the agonist NMDA (N-methyl-D-aspartate), is referred to as the NMDA type of EAA receptor. Two other glutamate-binding types of EAA receptor, which do not bind NMDA, are named according to their preference for binding with two other EAA receptor agonists, namely AMPA (alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionate), and kainate (2-carboxy-4-(1-methylethenyl)-3-pyrrolidineacetate). Accordingly, receptors which bind glutamate but not NMDA, and which bind with greater affinity to kainate than to AMPA, are referred to as kainate type EAA receptors. Similarly, those EAA receptors which bind glutamate but not NMDA, and which bind AMPA with greater affinity than kainate are referred to as AMPA type EAA receptors.

The glutamate-binding EAA receptor family is of great physiological and medical importance. Glutamate is involved in many aspects of long-term potentiation (learning and memory), in the development of synaptic plasticity, in epileptic seizures, in neuronal damage caused by ischemia following stroke or other hypoxic events, as well as in other forms of neurodegenerative processes. The development of therapeutics which modulate these processes has been very difficult, due to the lack of any homogeneous source of receptor material with which to discover selectively binding drug molecules, which interact specifically at the interface of the EAA receptor. The brain derived tissues currently used to screen candidate drugs are heterogeneous receptor sources, possessing on their surface many receptor types which interfere with studies of the EAA receptor/ligand interface of interest. The search for human therapeutics is further complicated by the limited availability of brain tissue of human origin. It would therefore be desirable to obtain cells that are genetically engineered to produce only the receptor of interest. With cell lines expressing cloned receptor cDNA, a substrate which is homogeneous for the desired receptor is provided, for drug screening programs.

Non-human cDNAs which appear to encode the NMDA-type of EAA receptor have recently been identified and isolated. A cDNA encoding a subunit polypeptide of an NMDA receptor in rat, designated NR1, has been isolated as described by Moriyoshi *et al.*, in *Nature* 354: 31, 1991. This work has been extended to demonstrate six isoforms of NR1, presumably generated by combinations of alternative RNA splicing in the amino- and carboxy-terminal regions of NR1 (Anantharam *et al.*, *FEBS Lett.* 305: 27, 1992; Durand *et al.*, *Proc. Natl. Acad. Sci. USA* 89: 9359, 1992; Nakanishi *et al.*, *Proc. Natl. Acad. Sci. USA* 89: 8552, 1992; Sugihara *et al.*, *Biochem. Biophys. Res. Commun.* 185: 826, 1992). DNA encoding NR1 and one of its isoforms have also been cloned from mouse brain by Yamazaki *et al.*, as described in *FEBS Lett.* 300: 39, 1992. Other rat NMDA receptor subunits, designated NR2A, NR2B and NR2C, have also been identified (Monyer *et al.*, *Science* 256: 1217, 1992), as well as mouse NMDA receptor subunits which have been designated ϵ 1, ϵ 2 and ϵ 3 (Meguro *et al.*, *Nature* 357: 70, 1992 and Kutsuwada *et al.*, *Nature* 358: 36, 1992).

There has emerged from these molecular cloning advances, a better understanding of the structural features of NMDA receptors and their subunits, as they exist in the non-human brain. According to the current model, each NMDA receptor is heteromeric, consisting of individual membrane-anchored subunits, each with four transmembrane regions, and extracellular domains that dictate ligand-binding properties and contribute to the ion-gating function served by the receptor complex.

In the search for therapeutics useful to treat CNS disorders in humans, it is highly desirable to obtain knowledge of human NMDA-type EAA receptors. A specific understanding of these human receptors would provide a means to screen for compounds that react therewith, i.e. to stimulate or inhibit receptor activity, and thus providing a means to identify compounds having potential therapeutic utility in humans. Non-human mammalian models are not suitable for this purpose despite significant receptor sequence homology, as minute

sequence discrepancies can cause dramatic pharmacological variation between species homologues of the same receptor (Oksenberg *et al.*, Nature, 360:161, 1992). It is therefore particularly desirable to provide cloned cDNA encoding human EAA receptors, and cell lines expressing these receptors in a homogeneous fashion, in order to generate a screening method for compounds therapeutically useful in humans. These, accordingly,

5 are objects of the present invention.

Another object of the present invention is to provide in isolated form a DNA molecule which codes for a human EAA receptor.

It is another object of the present invention to provide a cell that has been genetically engineered to produce an N-methyl-D-aspartate-type human EAA receptor.

10

Summary of the Invention

Human cDNAs encoding a family of EAA receptors, which bind glutamate with an affinity typical of EAA receptors and exhibit ligand binding properties characteristic of NMDA-type EAA receptors, have been identified and characterized. A representative member of this human EAA receptor family is herein designated human NMDAR1-1. Sequence-related cDNAs encoding naturally occurring variants of the human NMDAR1-1 receptor have also been identified, and constitute additional members of this receptor family as do fragments of NMDAR1 receptors, herein referred to as the human NMDAR1 receptor family.

15

The present invention thus provides, in one of its aspects, an isolated polynucleotide, consisting either of DNA or of RNA, which codes for a human NMDAR1 receptor, or for fragments thereof characterized by at least one of MK-801-binding or glutamate-binding.

20

In another aspect of the present invention, there is provided a cell that has been genetically engineered to produce a human EAA receptor belonging to the herein-defined NMDAR1 family. In related aspects of the present invention, there are provided recombinant DNA constructs and relevant methods useful to create such cells.

25

In another aspect of the present invention, there is provided a method for evaluating interaction between a test ligand and a human EAA receptor, which comprises the steps of incubating the test ligand with a genetically engineered cell of the present invention, or with a membrane preparation derived therefrom, and then assessing said interaction by determining receptor/ligand binding.

30

Other aspects of the present invention, which encompass various applications of the discoveries herein described, will become apparent from the following detailed description, and from the accompanying drawings in which:

Brief Reference to the Drawings

35

Figure 1 provides the nucleotide sequence of DNA coding for an excitatory amino acid receptor of the present invention, and the deduced amino acid sequence thereof;

Figure 2 illustrates with plasmid maps the strategy used to construct expression vectors harbouring the DNA sequence illustrated in Figure 1;

40

Figures 3-6 show, with reference to Figure 1, the DNA and amino acid sequences of naturally occurring variants of the EAA receptor illustrated in Figure 1; and

Figures 7 and 8 illustrate ligand-binding properties of the EAA receptor expressed from the coding region provided in Figure 1.

45

Detailed Description of the Invention and its Preferred Embodiments

The present invention relates to excitatory amino acid (EAA) receptors of human origin, and is directed more particularly to a novel family of NMDA-type human EAA receptors, herein designated the human NMDAR1 receptor family. NMDA-type human EAA receptors, generally designated herein as NMDA receptors, and including receptors of the NMDAR1 family, refer to those EAA receptors having specific binding affinity for glutamate and MK-801. NMDA is a competitive inhibitor of glutamate-binding while MK-801, the chemical formula for which is [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate], is a non-competitive antagonist of glutamate and has been shown to exhibit specific high-affinity binding to NMDA receptors. Consequently, NMDA receptors are characterized herein by both glutamate-binding and MK-801-binding as determined in assays of conventional design, such as the assays herein described.

55

As used herein, the term "human NMDAR1 receptor" is intended to embrace the mature human NMDAR1-1 receptor which demonstrates the typical ligand binding profile of an NMDA-type receptor, i.e. specific binding affinity for NMDA, glutamate and MK-801.

5 Variants of the NMDAR1 receptor are included within the meaning of "human NMDAR1 receptor" as defined above and include functional variants of the mature human NMDAR1 receptor which demonstrate the same ligand binding profile as the human NMDAR1 receptor, and which are structurally related thereto, i.e. share at least 99.6% amino acid identity with the 1-845 amino acid region of the NMDAR1-1 receptor, and preferably share 100% amino acid identity in this region. There are both naturally occurring and synthetically derived variants of the human NMDAR1 receptor. Naturally occurring variants include, but are not restricted to, the receptor variants of the human NMDAR1-1 receptor herein designated human NMDAR1-2, NMDAR1-3A, NMDAR1-3B, NMDAR1-3C, NMDAR1-4, NMDAR1-5, NMDAR1-6, NMDAR1-7 and NMDAR1-8. Synthetically derived variants of the human NMDAR1 receptor include variants of the parent NMDAR1 receptors, i.e. NMDAR1-1 and the naturally occurring variants thereof, which incorporate one or more e.g. 1 to 6 amino acid substitutions, deletions or additions, relative to the parent receptor.

10 The term "fragment" is used herein to denote segments of an NMDAR1 receptor which exhibit at least one of glutamate-binding or MK-801-binding. Since NMDAR1 receptors display specific binding for both glutamate and MK-801, the sites for glutamate and MK-801-binding are believed to be separate and distinct sites. Thus, fragments according to the present invention may display glutamate-binding, MK-801-binding, or both glutamate- and MK-801-binding.

The term "glutamate-binding", as it is used herein with respect to NMDAR1 receptors, and variants and fragments thereof, is meant to encompass those receptors, variants and fragments that display a greater binding affinity for glutamate than for NMDA, AMPA or kainate.

20 Further, the term "MK-801-binding", as it is used herein with respect to NMDAR1 receptors, and variants and fragments thereof, is meant to encompass those receptors, variants and fragments that display measurable binding for MK-801, for example, binding that is at least comparable to the binding of MK-801 to the NMDAR1-1 receptor. MK-801-binding is generally in the femtomolar range; however, preferably, MK-801-binding is greater than 10 femtomoles per milligram of protein as displayed herein by the NMDAR1-1 receptor.

25 Each of the naturally occurring members of the human NMDAR1 receptor family possesses structural features characteristic of EAA receptors in general, including extracellular amino- and carboxy-terminal regions, as well as four internal hydrophobic domains which serve to anchor the receptor within the cell surface membrane. The particular human EAA receptor designated NMDAR1-1 is a protein characterized structurally as a single polypeptide chain that is produced initially in precursor form bearing an 18 residue amino-terminal (N-terminal) signal peptide, and is transported to the cell surface in mature form, lacking the signal peptide and consisting of 867 amino acids arranged in the sequence illustrated, by single letter code, in Figure 1. Unless otherwise stated, the term "NMDAR1 receptor" refers to the mature form of the receptor protein, and amino acid residues of NMDAR1 receptors are accordingly numbered with reference to the mature protein sequence. With respect to structural domains of the receptor, hydropathy analysis reveals four putative transmembrane domains in NMDAR1-1, one spanning amino acid residues 544-562 inclusive (TM-1), another spanning residues 582-602 (TM-2), a third spanning residues 613-631 (TM-3) and a fourth spanning residues 795-815 (TM-4). Based on this assignment, it is likely that the human NMDAR1-1 receptor structure, in its natural membrane-bound form, consists of a 543 amino acid N-terminal extracellular domain, followed by a hydrophobic region containing four transmembrane domains and an extracellular, 52 amino acid carboxy-terminal (C-terminal) domain.

40 As shown in Figures 3 to 6, nine structurally-related variants of the NMDAR1-1 receptor have also been identified and are designated as set out above. As deduced from the cDNAs encoding these receptors, the nucleotide sequence of NMDAR1-2 comprises the NMDAR1-1 nucleotide sequence and further includes a 363 bp insert between nucleotides 3687 and 3688 (Figure 3A). The nucleotide sequences of NMDAR1-3A, NMDAR1-3B and NMDAR1-3C comprise the NMDAR1-1 nucleotide sequence and further include a 474 bp insert between nucleotides 3687 and 3688. These insertions introduce a new open reading frame and TGA stop codon, and as a result, the C-terminal amino acid sequence subsequent to amino acid residue 845 in the variant receptors is very different from the C-terminus of NMDAR1-1 (Fig. 3B). The mature human NMDAR1-2 and NMDAR1-3A, 3B and 3C receptors consist of 883 and 920 amino acids, respectively. The amino acid sequences of NMDAR1-3A and NMDAR1-3C differ by a single amino acid residue due to a base pair change in the nucleotide sequence of the 3A variant. The codon at position 217 of the inserted region in the 3A variant is changed from AGG to ACG in the 3C variant. This codon change alters the amino acid encoded from arginine in NMDAR1-3A to threonine in NMDAR1-3C. The amino acid sequence of NMDAR1-3B differs from the amino acid sequences of NMDAR1-1 and NMDAR1-3C by a single amino acid at position 470 in which the lysine of NMDAR1-3B is glutamic acid in NMDAR1-1 and NMDAR1-3C. This results from a single base pair change in the codon at position 2560 of NMDAR1-1 and NMDAR1-3C from GAG to AAG in the 3B variant (Fig. 4). The NMDAR1-4 receptor is encoded by a nucleotide sequence corresponding to that of NMDAR1-1 which further includes a 111 bp insert between nucleotides 3687 and 3688 (Figure 5A) which encodes a peptide insert be-

tween amino acids 845 and 846 of NMDAR1-1 (Figure 5B). The mature NMDAR1-4 protein comprises 904 amino acids. The NMDAR1-5, NMDAR1-6, NMDAR1-7 and NMDAR1-8 variants correspond respectively to the NMDAR1-1, NMDAR1-2, NMDAR1-3 and NMDAR1-4 receptors additionally including a 63 bp insertion at their N-terminal end between nucleotides 1663 and 1664 (Figure 6A). The amino acid sequence of this insertion is illustrated in Figure 6B.

In human hippocampus cDNA libraries, the source from which DNA coding for the NMDAR1-1 receptor was isolated, the NMDAR1-1 receptor is encoded by the nucleotide sequence provided in Figure 1; however, due to the degeneracy associated with nucleotide triplet codons, it will be appreciated that the NMDAR1 receptor may be encoded by polynucleotides incorporating codons synonymous with those illustrated in Figure 1. For example, as would be known by one of skill in the art, arginine may be encoded by any one of six codons selected from CGA, CGC, CGG, CGU, AGA and AGG, threonine may be encoded by any one of four codons selected from ACA, ACC, ACG and ACU, while lysine is encoded by two codons, AAA and AAG.

Like other members of the human NMDAR1 receptor family, receptor subtype NMDAR1-1 is characterized by a pharmacological profile i.e. a ligand binding "signature", that points strongly to an NMDA-type EAA receptor as distinct from other excitatory amino acid receptor types, such as AMPA and kainate receptors. In addition, and despite the understanding that NMDA-type receptors require a multi- and perhaps heteromeric subunit structure to function in the pharmacological sense, it has been found that cells producing the unitary NMDAR1-1 receptor do, independently of association with other receptor subunits, provide a reliable indication of excitatory amino acid binding. Thus, in a key aspect of the present invention, the human NMDAR1-1 receptor and the variants thereof, are exploited for the purpose of screening candidate compounds for the ability to interact with the present receptors and/or the ability to compete with endogenous EAA receptor ligands and known synthetic analogues thereof.

For use in assessing interaction between the receptor and a test ligand, it is desirable to construct by application of genetic engineering techniques a cell that produces a human NMDAR1 receptor in functional form as a heterologous product. The construction of such cell lines is achieved by introducing into a selected host cell a recombinant DNA construct in which DNA coding for a secretable form of the human NMDAR1 receptor, i.e. a form bearing either its native signal peptide or a functional, heterologous equivalent thereof, is associated with expression controlling elements that are functional in the selected host to drive expression of the receptor-encoding DNA, and thus elaborate the desired NMDAR1 receptor protein. Such cells are herein characterized as having the receptor-encoding DNA incorporated "expressibly" therein. The receptor-encoding DNA is referred to as "heterologous" with respect to the particular cellular host if such DNA is not naturally found in the particular host.

It is most desirable to use a mammalian cell host to produce NMDAR1 receptors due to the mammalian origin of the present human NMDAR1 receptors; however, other suitably engineered eukaryotic and prokaryotic hosts may also be employed to produce NMDAR1 receptors. Accordingly, bacterial hosts such as *E. coli* and *B. subtilis*, fungal hosts such as *Aspergillus* and yeast and insect cell hosts such as *Spodoptera frugiperda*, are examples of non-mammalian hosts that may also be used to produce NMDAR1 receptors of the present invention.

The particular cell type selected to serve as host for production of the human NMDAR1 receptor can be any of several cell types currently available in the art, but should not of course be a cell type that in its natural state elaborates a surface receptor that can bind excitatory amino acids, and so confuse the assay results sought from the engineered cell line. Generally, such problems are avoided by selecting as host a non-neuronal cell type, and can further be avoided using non-human cell lines, as is conventional. It will be appreciated that neuronal- and human-type cells may nevertheless serve as expression hosts, provided that "background" binding to the test ligand is accounted for in the assay results.

According to one embodiment of the present invention, the cell line selected to serve as host for NMDAR1 receptor production is a mammalian cell. Several types of such cell lines are currently available for genetic engineering work, and these include the chinese hamster ovary (CHO) cells for example of K1 lineage (ATCC CCL 61) including the Pro5 variant (ATCC CRL 1281); fibroblast-like cells derived from SV40-transformed African Green monkey kidney of the CV-1 lineage (ATCC CCL 70), of the COS-1 lineage (ATCC CRL 1650) and of the COS-7 lineage (ATCC CRL 1651); murine L-cells, murine 3T3 cells (ATCC CRL 1658), murine C127 cells, human embryonic kidney cells of the 293 lineage (ATCC CRL 1573), human carcinoma cells including those of the HeLa lineage (ATCC CCL 2), and neuroblastoma cells of the lines IMR-32 (ATCC CCL 127), SK-N-MC (ATCC HTB 10) and SK-N-SH (ATCC HTB 11).

A variety of gene expression systems have been adapted for use with these hosts and are now commercially available. Any one of these systems can be exploited to drive expression of the NMDAR1 receptor-encoding DNA. These systems, available typically in the form of plasmidic vectors, incorporate expression cassettes, the functional components of which include DNA constituting host-recognizable expression controlling

sequences which enable expression of the receptor-encoding DNA when linked 5' thereof. The systems further incorporate DNA sequences which terminate expression when linked 3' of the receptor-encoding region. Thus, for expression in a selected mammalian cell host, there is generated a recombinant DNA expression construct in which DNA encoding an NMDAR1 receptor is linked with expression controlling DNA sequences recognized by the host, including a region 5' of the receptor-encoding DNA to drive expression, and a 3' region to terminate expression. The plasmidic vector harbouring the expression construct typically incorporates such other functional components as an origin of replication, usually virally-derived, to permit replication of the plasmid in the expression host, including bacterial hosts such as *E. coli*. To provide a marker enabling selection of stably transformed recombinant cells, the vector will also incorporate a gene conferring some survival advantage on the transformants, such as a gene coding for neomycin resistance in which case the transformants are plated in medium supplemented with neomycin.

Included among the various recombinant DNA expression systems that can be used to achieve mammalian cell expression of the receptor-encoding DNA are those that exploit promoters of viruses that infect mammalian cells, such as the promoter from the cytomegalovirus (CMV), the Rous sarcoma virus (RSV), simian virus (SV40), murine mammary tumor virus (MMTV) and others. Also useful to drive expression are promoters such as the long terminal repeat (LTR) of retroviruses, insect cell promoters such as those regulated by temperature, and isolated from *Drosophila*, as well as mammalian gene promoters such as steroid-inducible promoters and those regulated by heavy metals i.e. the metallothionein gene promoter. In order to achieve expression in bacterial hosts, such as *E. coli*, expression systems that exploit the expression controlling regions of various *E. coli* and viral genes can be used to drive NMDAR1 receptor production including the lac gene, the trp gene, and regions of the lambda genome (PL and PR). Expression in yeast can be achieved using the expression-controlling regions of genes such as alcohol dehydrogenase and melibiase, and in *Aspergillus*, the expression-controlling regions of genes such as alcohol dehydrogenase and glucoamylase may be used. The expression controlling-regions of baculovirus may be used in the case of insect host cells.

For incorporation into the recombinant DNA expression vector, DNA coding for the desired NMDAR1 receptor, e.g. the NMDAR1-1 receptor, an MK-801-binding variant thereof, or a variant of the NMDAR1-1 receptor, can be obtained by applying selected techniques of gene isolation or gene synthesis. As described in more detail in the examples herein, the NMDAR1-1 receptor, and variants thereof, are encoded within the genome of human brain tissue, and can therefore be obtained by careful application of conventional gene isolation and cloning techniques. This typically will entail extraction of total messenger RNA from a fresh source of human brain tissue, such as cerebellum or fetal brain tissue and preferably hippocampus tissue, followed by conversion of message to cDNA and formation of a library in, for example, a bacterial plasmid, or more typically a bacteriophage. Bacteriophage harbouring fragments of the human DNA are typically grown by plating on a lawn of susceptible *E. coli* bacteria, such that individual phage plaques or colonies can be isolated. The DNA carried by the phage colony is then typically immobilized on a nitrocellulose or nylon-based hybridization membrane, and then hybridized, under carefully controlled conditions, to a radioactively (or otherwise) labelled nucleotide probe of appropriate sequence to identify the particular phage colony carrying receptor-encoding DNA or fragments thereof. Typically, the gene or a portion thereof so identified is subcloned into a plasmidic vector for nucleic acid sequence analysis.

Having herein provided the nucleotide sequence of various human NMDAR1 receptors, it will be appreciated that automated techniques of gene synthesis and/or amplification can be performed to generate DNA coding therefor. Because of the length of NMDAR1 receptor-encoding DNA, application of automated synthesis may require staged gene construction, in which regions of the gene up to about 300 nucleotides in length are synthesized individually and then ligated in correct succession for final assembly. Individually synthesized gene regions can be amplified prior to assembly using polymerase chain reaction (PCR) technology as generally described by Barnett *et al.* in *Nucl. Acids Res.* 18:3094, 1990.

The application of automated gene synthesis techniques provides an opportunity to generate sequence variants of naturally occurring members of the NMDAR1 gene family. It will be appreciated, for example and as mentioned above, that polynucleotides coding for the NMDAR1 receptors herein described can be generated by substituting synonymous codons for those represented in the naturally occurring polynucleotide sequences herein identified. In addition, polynucleotides coding for synthetic variants of the NMDAR1 receptors herein described can be generated which, for example, incorporate one or more single amino acid substitutions, deletions or additions. Since it will for the most part be desirable to retain the natural ligand binding profile of the receptor for screening purposes, it is desirable to limit amino acid substitutions to the so-called conservative replacements in which amino acids of like charge are substituted, and to limit substitutions to those regions which are less critical for receptor activity as may be elucidated upon receptor domain mapping.

With appropriate template DNA in hand, the technique of PCR amplification may also be used to directly generate all or part of the final gene. In this case, primers are synthesized which will prime the PCR amplifi-

cation of the final product, either in one piece, or in several pieces that may be ligated together. This may be via step-wise ligation of blunt-ended, amplified DNA fragments, or preferentially via step-wise ligation of fragments containing naturally occurring restriction endonuclease sites. In this application, it is possible to use either cDNA or genomic DNA as the template for the PCR amplification. In the former case, the cDNA template can be obtained from commercially available or self-constructed cDNA libraries of various human brain tissues, including hippocampus and cerebellum.

Once obtained, the receptor-encoding DNA is incorporated for expression into any suitable expression vector using conventional procedures, and host cells are transfected therewith also using conventional procedures which include, for example, DNA-mediated transformation, electroporation, microinjection, or particle gun transformation. Expression vectors may be selected to provide transformed mammalian cell lines that express the receptor-encoding DNA either transiently or in a stable manner. For transient expression, host cells are typically transformed with an expression vector harbouring an origin of replication functional in a mammalian cell. For stable expression, such replication origins are unnecessary, but the vectors will typically harbour a gene coding for a product that confers on the transformants a survival advantage, to enable their selection. Genes coding for such selectable markers include, but are not limited to, the *E. coli gpt* gene which confers resistance to mycophenolic acid, the *neo* gene from transposon Tn5 which confers resistance to the antibiotic G418 and to neomycin, the *dhfr* sequence from murine cells or *E. coli* which changes the phenotype of DHFR-cells into DHFR+ cells, and the *tk* gene of herpes simplex virus, which makes TK- cells phenotypically TK+ cells. Both transient expression and stable expression can provide transformed cell lines, and membrane preparations derived therefrom, for use in ligand screening assays.

For use in screening assays, cells transiently expressing the receptor-encoding DNA can be stored frozen for later use, but because the rapid rate of plasmid replication will lead ultimately to cell death, usually in a few days, the transformed cells should be used as soon as possible. Such assays may be performed either with intact cells, or with membrane preparations derived from such cells. The membrane preparations typically provide a more convenient substrate for the ligand binding experiments, and are therefore preferred as binding substrates. To prepare membrane preparations for screening purposes, i.e. ligand binding experiments, frozen intact cells are homogenized while in cold water suspension and a membrane pellet is collected after centrifugation. The pellet is re-suspended and re-centrifuged to remove endogenous EAA ligands such as glutamate, that would otherwise compete for binding in the assays. The membranes may then be used as such, or after storage in lyophilized form, in the ligand binding assays. Alternatively, intact, fresh cells harvested about two days after transient transfection or after about the same period following fresh plating of stably transfected cells, can be used for ligand binding assays by the same methods as used for membrane preparations. When cells are used, the cells must be harvested by more gentle centrifugation so as not to damage them, and all washing must be done in a buffered medium, for example in phosphate-buffered saline, to avoid osmotic shock and rupture of the cells.

The binding of a candidate ligand to a selected human NMDAR1 receptor of the invention is evaluated typically using a predetermined amount of cell-derived membrane (measured for example by protein determination), generally from about 25 ug to 100 ug. Generally, competitive binding assays will be useful to evaluate the affinity of a test compound relative to glutamate, for the receptor. This competitive binding assay can be performed by incubating the membrane preparation with radiolabelled glutamate, for example [³H]-glutamate, in the presence of unlabelled test compound added at varying concentrations. Following incubation, either displaced or bound radiolabelled glutamate can be recovered and measured to determine the relative binding affinities of the test compound and glutamate for the particular receptor used as substrate. In this way, the affinities of various compounds for the NMDA-type human EAA receptors can be measured.

The NMDAR1 receptors of the present invention are per se functional in an electrophysiological context, and are therefore useful, in the established manner, in screening test ligands for their ability to modulate ion channel activity. The present invention thus further provides, as a ligand screening technique, a method of detecting interaction between a test ligand and a human CNS receptor, which comprises the steps of incubating the test ligand with a human NMDAR1 receptor-producing cell or with a membrane preparation derived therefrom, and then measuring ligand-induced electrical current across said cell or membrane.

As an alternative to using cells that express receptor-encoding DNA, ligand characterization, either through binding or through ion channel formation, may also be performed using cells (for example *Xenopus* oocytes), that yield functional membrane-bound receptor following introduction of messenger RNA coding for the NMDAR1 receptor. In this case, NMDAR1 receptor DNA is typically subcloned into a plasmidic vector such that the introduced DNA may be easily transcribed into RNA via an adjacent RNA transcription promoter supplied by the plasmidic vector, for example the T3 or T7 bacteriophage promoters. RNA is then transcribed from the inserted gene *in vitro*, and isolated and purified therefrom for injection into *Xenopus* oocytes. Following the injection of nanoliter volumes of an RNA solution, the oocytes are left to incubate for up to several days,

and are then tested for the ability to respond to a particular ligand molecule supplied in a bathing solution. Since functional EAA receptors act in part by operating a membrane channel through which ions may selectively pass, the functioning of the receptor in response to a particular ligand molecule in the bathing solution may typically be measured as an electrical current utilizing microelectrodes inserted into the cell or placed on either side of a cell-derived membrane preparation using the "patch-clamp" technique.

In addition to using the receptor-encoding DNA to construct cell lines useful for ligand screening, expression of the DNA can, according to another aspect of the invention, be performed to produce fragments of the receptor in soluble form, for structure investigation, to raise antibodies and for other experimental uses. It is expected that the portion of the NMDAR1 receptor responsible for binding a ligand molecule resides on the outside of the cell, i.e. is extracellular. It is therefore desirable in the first instance to facilitate the characterization of the receptor-ligand interaction by providing this extracellular ligand-binding domain in quantity and in isolated form, i.e. free from the remainder of the receptor. To accomplish this, the full-length NMDAR1 receptor-encoding DNA may be modified by site-directed mutagenesis, to introduce a translational stop codon into the extracellular N-terminal region, immediately 5' of the first transmembrane domain (TM1), i.e., before the amino acid residue 544 codon as shown in Figure 1. Since there will no longer be produced any transmembrane domain(s) to "anchor" the receptor into the membrane, expression of the modified cDNA will result in the secretion, in soluble form, of only the extracellular N-terminal ligand-binding domain. Standard ligand-binding assays may then be performed to ascertain the degree of binding of a candidate compound to the extracellular domain so produced. It may of course be necessary, using site-directed mutagenesis, to produce different versions of the extracellular regions, in order to map the ligand binding domain with precision. It will also be appreciated that the length of the fragment may be varied, i.e. to lengths less than the entire 544 amino acid extracellular N-terminal domain.

Alternatively, it may be desirable to produce an extracellular domain of the receptor which is not derived from the N-terminus of the mature protein, but rather from the carboxy-terminus, for example domains immediately following the fourth transmembrane domain (TM4), e.g. residing between amino acid residues 816 and 867 inclusive in NMDAR1-1 as shown in Figure 1, between amino acid residues 816 and 883 in NMDAR1-2 or between amino acid residues 816 and 920 in NMDAR1-3A, -3B and -3C. In this case, site-directed mutagenesis and/or PCR-based amplification techniques may readily be used to provide a defined fragment of the cDNA encoding the receptor domain of interest. Direct peptide synthesis may also be used to make the desired C-terminal fragment, or as noted above, desired N-terminal fragments. Such a DNA sequence may be used to direct the expression of the desired receptor fragment, either intracellularly, or in secreted fashion, provided that the DNA encoding the gene fragment is inserted adjacent to a translation start codon provided by the expression vector, and that the required translation reading frame is carefully conserved.

It will be appreciated that the production of such extracellular ligand binding domains may be accomplished in a variety of host cells. Mammalian cells such as CHO cells may be used for this purpose, the expression typically being driven by an expression promoter capable of high-level expression, for example, the CMV promoter. Alternately, non-mammalian cells, such as insect Sf9 (*Spodoptera frugiperda*) cells may be used, with the expression typically being driven by expression promoters of the baculovirus, for example the strong, late polyhedrin protein promoter. Filamentous fungal expression systems may also be used to secrete large quantities of such extracellular domains of the NMDAR1 receptor. *Aspergillus nidulans* for example, with the expression being driven by the *alcA* promoter, would constitute such an acceptable fungal expression system. In addition to such expression hosts, it will be further appreciated that any prokaryotic or other eukaryotic expression system capable of expressing heterologous genes or gene fragments, whether intracellularly or extracellularly would be similarly acceptable.

For use particularly in detecting the presence and/or location of a NMDAR1 receptor, for example in brain tissue, the present invention also provides, in another of its aspects, labelled antibody to a human NMDAR1 receptor. To raise such antibodies, there may be used as immunogen either the intact, soluble receptor or an immunogenic fragment thereof, produced in a microbial or mammalian cell host as described above or by standard peptide synthesis techniques. Regions of the NMDAR1-1 receptor particularly suitable for use as immunogenic fragments include those corresponding in sequence to an extracellular region of the receptor, or a portion of the extracellular region, such as peptides consisting of residues 1-543, including particularly residues 497-539, and peptides corresponding to the extracellular region between transmembrane domains TM-2 and TM-3, such as a peptide consisting of residues 603-612. Peptides consisting of the carboxy-terminal domain (residues 816-867), or fragments thereof may also be used for the raising of antibodies. Substantially the same regions of the variants of human NMDAR1-1, namely, the NMDAR1-2 to NMDAR1-8 receptors, may also be used for production of antibodies, taking into account the elongated carboxy terminal domains of a number of these variants.

The raising of antibodies to the desired NMDAR1 receptor or immunogenic fragment can be achieved, for

polyclonal antibody production, using immunization protocols of conventional design, and any of a variety of mammalian hosts, such as sheep, goats and rabbits. Alternatively, for monoclonal antibody production, immunocytes such as splenocytes can be recovered from the immunized animal and fused, using hybridoma technology, to myeloma cells. The fusion cell products, i.e. hybridoma cells, are then screened by culturing in a selection medium, and cells producing the desired antibody are recovered for continuous growth, and antibody recovery. Recovered antibody can then be coupled covalently to a reporter molecule, i.e. a detectable label, such as a radiolabel, enzyme label, luminescent label or the like, using linker technology established for this purpose, to form a specific probe for NMDAR1 receptors.

In detectably labelled form, e.g. radiolabelled form, DNA or RNA coding for the human NMDAR1 receptor, and selected regions thereof, may also be used, in accordance with another aspect of the present invention, as hybridization probes for example to identify sequence-related genes resident in the human or other mammalian genomes (or cDNA libraries) or to locate the NMDAR1-encoding DNA in a specimen, such as brain tissue. This can be done using either the intact coding region, or a fragment thereof, having radiolabelled nucleotides, for example, ^{32}P nucleotides, incorporated therein. To identify the NMDAR1-encoding DNA in a specimen, it is desirable to use either the full length cDNA coding therefor, or a fragment which is unique thereto. With reference to Figure 1 and the nucleotide numbering appearing thereon, such nucleotide fragments include those comprising at least about 17 nucleic acids, and otherwise corresponding in sequence to a region coding for the N-terminus or C-terminus of the receptor, or representing a 5'-untranslated or 3'-untranslated region thereof. One example of a suitable nucleotide fragment is the region spanning nucleotides 2605 to 3213 of NMDAR1-1, as described herein in the Examples. These sequences, and the intact gene itself, may also be used of course to clone NMDAR1-related human genes, particularly cDNA equivalents thereof, by standard hybridization techniques.

Embodiments of the present invention are described in detail in the following non-limiting Examples.

Example 1 Isolation of DNA coding for the human NMDAR1-1 receptor

A human NMDAR1 probe corresponding to a portion of nucleotide sequence of NMDAR1-1, namely the nucleotide region 2605-3213 as shown in Fig. 1, was generated by PCR-based amplification of recombinant bacteriophage lambda DNA isolated from an Eco RI-based bacteriophage λ library of human hippocampus cDNA (obtained from Stratagene Cloning Systems, La Jolla, CA.). The following degenerate oligonucleotide primers were used in the PCR amplification:

1) 5' GGGGTTTGGATCCAA-A/G-GA-A/G-TGGAA-C/T-GGNATGATG 3';

and

2) 5' GGGGTTTAAGCTT-C/T-TC-G/A-TA-G/A-TT-G/A-TG-C/T-TT-C/T-TCCAT 3'

The primers were used at a final concentration of 5 pmol/ul each, in a 50 ul reaction volume (10 mM Tris-HCl, pH 9.0; 50 mM KCl; 1.5 mM MgCl_2) containing 100 ng of recombinant human hippocampus cDNA/bacteriophage λ DNA, 5 units of *Thermus aquaticus* DNA polymerase (Promega, Madison, WI.) and 0.2 mM of each deoxyribonucleotide. Thirty-five cycles of amplification proceeded, with denaturation at 94°C for 1 min., annealing at 51°C for 1 min., and primer extension at 72°C for 1 min., followed by a final cycle at 72°C for 5 min. The 674 bp PCR product was purified from an agarose gel and subcloned into the plasmid vector pTZBlue-T (Novagen, Madison, WI.) for DNA sequencing. The nucleotide sequence of this fragment was 88% identical to that of the rat NMDAR1 cDNA.

The 674 bp human NMDAR1 probe was radiolabelled with [α - ^{32}P]dCTP using the Amersham Megaprime DNA labelling system (Arlington Heights, IL.) to a specific activity of $1.0\text{--}2.4 \times 10^9$ cpm/ug. The labelled probe was used to screen approximately 400,000 plaques of an Eco RI-based human hippocampus cDNA/ bacteriophage λ Zap II library. Thirty-five positive plaques were identified on replica filters under the following hybridization conditions: 6X SSC, 50% formamide, 0.5% SDS, 100 ug/ml denatured salmon sperm DNA at 42°C with 1.85×10^6 cpm probe per ml hybridization fluid. The filters were washed with 2X SSC, 0.5% SDS at 25°C for 5 min., followed by 15 min. washes at 37°C and at 42°C. The filters were exposed to X-ray film (Kodak, Rochester, NY.) overnight. Twenty-eight plaques were purified and excised as phagemids according to the supplier's specifications, to generate an insert-carrying Bluescript-SK variant of the phagemid vector.

DNA sequence analysis of the largest clone (NMDAR1-1) revealed a putative ATG initiation codon together with about 1098 bases of 5' non-coding information and 2655 bases of amino acid coding information. This analysis also revealed a termination codon as well as about 906 bases of 3' non-translated information. The entire DNA sequence of the EcoRI-EcoRI NMDAR1-1 cDNA insert is provided in Figure 1.

A 7.6 kb phagemid designated pBS/humNMDAR1-1 carrying the receptor-encoding DNA as a 4.7 kbp EcoRI-EcoRI insert in a 2.9 kbp Bluescript-SK-phagemid background, was deposited, under the terms of the Budapest Treaty, with the American Type Culture Collection in Rockville, Maryland, USA on November 12, 1992 and has been assigned accession number ATCC 75349.

Example 2 Construction of genetically engineered cells producing the human NMDAR1-1 receptor

For transient expression in mammalian cells, cDNA coding for the human NMDAR1-1 receptor was incorporated into the mammalian expression vector pcDNA1/Amp, which is available commercially from Invitrogen Corporation (San Diego, CA.). This is a multifunctional 4.8 kbp plasmid vector designed for cDNA expression in eukaryotic systems, and cDNA analysis in prokaryotes. Incorporated on the vector are the CMV immediate early gene promoter and enhancer sequences, SV40 transcription termination and RNA processing signals, SV40 and polyoma virus origins of replication, M13 and ColE1 origins, Sp6 and T7 RNA promoters, and a gene conferring ampicillin resistance. A polylinker is located appropriately downstream of the CMV and T7 promoters.

The strategy depicted in Figure 2 was employed to facilitate incorporation of the NMDAR1-1 receptor-encoding cDNA into the expression vector. The cDNA insert was released from pBS/humNMDAR1-1 as a 4.7 kbp Sal I/Spe I fragment, which was then incorporated at the Xho I/Xba I sites in the pcDNA1/Amp polylinker. DNA sequence analysis across the junctions was performed to confirm proper insert orientation. The resulting plasmid, designated pcDNA1/Amp/humNMDAR1-1, was then introduced for transient expression into a selected mammalian cell host, in this case the monkey-derived, fibroblast-like cells of the COS-1 lineage (available from the American Type Culture Collection, Rockville, Maryland as ATCC CRL 1650).

For transient expression of the humNMDAR1-1-encoding DNA, COS-1 cells were transfected with approximately 8ug DNA (as pcDNA1/Amp/humNMDAR1-1) per 10^6 COS-1 cells, by DEAE-mediated DNA transfection and treated with chloroquine according to the procedures described by Sambrook *et al.*, Molecular Cloning-A Laboratory Manual, Cold Spring Harbour Laboratory Press, 1989. Briefly, COS-1 cells were plated at a density of 5×10^6 cells/dish and then grown for 24 hours in 10% FBS-supplemented DMEM/F12 medium. Medium was then removed and cells were washed in PBS followed by medium (lacking FBS). Ten milliliters of a transfection solution containing DEAE dextran (0.4mg/ml), 100uM chloroquine, 10% NuSerum, DNA (0.4mg/ml) in DMEM/F12 medium was then applied to the cells. After incubation for 3 hours at 37°C, cells were washed as previously described and then shocked for 1 minute with 10% DMSO in DMEM/F12 medium. Cells were allowed to grow for 2-3 days in 10% FBS-supplemented medium, and at the end of incubation, dishes were placed on ice, the cells were washed with ice cold PBS and then removed by scraping. Cells were then harvested by centrifugation at 1000 rpm for 10 minutes and the cellular pellet was frozen in liquid nitrogen for subsequent use in ligand binding assays.

In a like manner, stably transfected cell lines can also be prepared using two different cell types as host: CHO K1 and CHO Pro5. To construct these cell lines, cDNA coding for human NMDAR1 is incorporated into the mammalian expression vector pRC/CMV (Invitrogen) which enables stable expression. Insertion of the cDNA places it under the expression control of the CMV promoter and upstream of the polyadenylation site and terminator of the bovine growth hormone gene, and into a vector background comprising the neomycin resistance gene (driven by the SV40 early promoter) as selectable marker.

To introduce plasmids constructed as described above, the host CHO cells are first seeded at a density of 5×10^5 cells/dish in 10% FBS-supplemented α MEM medium. After growth for 24 hours, fresh medium is added to the plates and three hours later, the cells are transfected using the calcium phosphate-DNA co-precipitation procedure (Sambrook *et al. supra*). Briefly, 3 ug of DNA is mixed and incubated with buffered calcium solution for 10 minutes at room temperature. An equal volume of buffered phosphate solution is added and the suspension is incubated for 15 minutes at room temperature. Next, the incubated suspension is applied to the cells for 4 hours, removed and cells were shocked with medium containing 15% glycerol. Three minutes later, cells are washed with medium and incubated for 24 hours at normal growth conditions. Cells resistant to neomycin are selected in 10% FBS-supplemented α -MEM medium containing G418 (1mg/ml). Individual colonies of G418-resistant cells are isolated about 2-3 weeks later, clonally selected and then propagated for assay purposes.

Example 3 - Ligand binding assays

Frozen transfected COS cells were resuspended in ice-cold distilled water, sonicated for 5 seconds, and centrifuged for 10 minutes at 50,000 x g. The supernatant was discarded and the membrane pellet stored frozen at - 70°C.

COS-1 cell membrane pellets were resuspended in ice cold 50 mM Tris-HCl, pH 7.55, and centrifuged again at 50,000 x g for 10 minutes in order to remove endogenous glutamate that would otherwise compete for binding. The pellets were resuspended in ice cold 50 mM Tris-HCl, pH 7.55, and used for the binding experiments described below. Protein concentrations were determined using the Pierce reagent with BSA as an internal standard.

Binding assays were performed using a 25-100 µg protein equivalent of the COS-derived membrane preparation, and a selected radiolabelled ligand. In particular, for MK-801-binding assays, incubation mixtures consisted of 20 nM (+)-[3-³H]MK-801 (30 Ci/mmmole), 20 µM glycine, and 1 mM L-glutamate in the cold incubation buffer at a final volume of 250 µl. Non-specific binding was determined in the presence of 1 mM (+)MK-801. For glutamate binding assays, incubation mixtures consisted of 30 nM [3,4-³H]-L-glutamate (47.3 Ci/mmmole) in the cold incubation buffer at a final volume of 250 µl. Non-specific binding was determined in the presence of 1 mM L-glutamate and displacement was determined in the presence of 1 mM NMDA, 1 mM kainate, or 1 mM AMPA. The reaction mixtures were incubated on ice for 60 minutes in plastic mini-vials. Bound and free ligand were separated by centrifugation for 30 minutes at 50,000 x g. The pellets were washed three times in 4 ml of the cold incubation buffer, and then 4 ml of Beckman Ready-Protein Plus scintillation cocktail was added for liquid scintillation counting.

Assays performed in this manner, using membrane preparations derived from the human NMDAR1-1-producing COS-1 cells, revealed specific [³H]MK-801 binding at 20nM labelled ligand (Figure 7), and specific [³H]-L-glutamate binding at 30 nM labelled ligand (Figure 8). The level of specific binding for MK-801 was determined to be 1286 fmol/mg protein, and the specific binding for glutamate was determined to be 387 fmol/mg protein. Mock transfected cells exhibited no specific binding of any of the ligands tested. Some displacement of [³H]-glutamate binding could be observed in the presence of 1 mM NMDA. These results demonstrate clearly that the human NMDAR1-1 receptor is binding glutamate and MK-801 specifically. This property clearly assigns the human NMDAR1-1 receptor to be of the NMDA-type of EAA receptor. Furthermore, this binding profile indicates that the receptor is functioning in an authentic manner, and can therefore be used to reliably predict the ligand binding "signature" of its non-recombinant counterpart from the intact human brain. These features make the recombinant receptor especially useful for selecting and characterizing ligand compounds which bind to the receptor, and/or for selecting and characterizing compounds which may act by displacing other ligands from the receptor. The isolation of the NMDAR1-1 receptor gene in a pure form, capable of being expressed as a single, homogenous receptor species, therefore frees the ligand binding assay from the lack of precision introduced when complex, heterogeneous receptor preparations from human and non-human brains are used to attempt such characterizations.

Example 4 - Isolation and Cloning of NMDAR1-1 Variant Receptors

The procedures described in Examples 1 and 2 for isolating and cloning the NMDAR1-1 receptor apply equally to the naturally occurring variant receptors of NMDAR1-1, particularly in view of sequence similarities between the NMDAR1-1 receptor and the identified variants thereof.

Moreover, the ligand-binding assay set out in Example 3 can be used in the manner described to determine the ligand binding characteristics of receptor variants.

Claims

1. An isolated polynucleotide comprising a nucleotide sequence that codes for a human NMDAR1 receptor, or for a fragment thereof which exhibits at least one of MK-801-binding or glutamate-binding.
2. An isolated polynucleotide according to claim 1, wherein said nucleotide sequence codes for a receptor selected from the human NMDAR1-1, NMDAR1-2, NMDAR1-3A, NMDAR1-3B, NMDAR1-3C, NMDAR1-4, NMDAR1-5, NMDAR1-6, NMDAR1-7 and NMDAR1-8 receptors, or a fragment thereof which exhibits at least one of MK-801-binding or glutamate-binding.
3. An isolated polynucleotide encoding a variant of a human NMDAR1 receptor, wherein said variant shares

at least 99.6% amino acid identity with the 1-845 amino acid region of the NMDAR1-1 receptor.

4. A recombinant DNA construct having incorporated therein a polynucleotide as defined in any one of claims 1-3.
5. A cell that has been engineered genetically to produce a human NMDAR1 receptor or a fragment thereof, said cell having incorporated expressibly therein a heterologous polynucleotide as defined in any one of claims 1 to 3.
6. A cell as defined in claim 5, which is a mammalian cell.
7. A membrane preparation derived from a cell as defined in claim 5.
8. A process for obtaining a substantially homogeneous source of a human EAA receptor, which comprises the steps of culturing cells having incorporated expressibly therein a polynucleotide as defined in any one of claims 1-3, and then recovering the cultured cells.
9. A process for obtaining a substantially homogeneous source of a human EAA receptor according to claim 8, comprising the subsequent step of obtaining a membrane preparation from the cultured cells.
10. A method of assaying a test ligand for interaction with a human CNS receptor, which comprises the steps of incubating the test ligand under appropriate conditions with a human NMDAR1 receptor-producing cell as defined in claim 5 or with membrane preparation derived therefrom, and then determining the extent of binding between the human NMDAR1 receptor and the test ligand, or the ligand-induced electrical current across said cell or membrane.
11. A human NMDAR1 receptor, in a form essentially free from other proteins of human origin.
12. An MK-801-binding or glutamate-binding fragment of a human NMDAR1 receptor.
13. An antibody which binds a human NMDAR1 receptor.
14. An immunogenic fragment of a human NMDAR1 receptor.
15. An oligonucleotide comprising at least about 17 nucleic acids which hybridizes with a polynucleotide defined in claim 1.

FIGURE 1

Eco RI

GAATTCGGTAAGGCTCTGGAAAAGGGGGCGCTGGGAGCGCATTGCGAGGGGGCTGGAGA
 -----+-----+-----+-----+-----+-----+ 60
 CTTAAGGCCATTCCGAGACCTTTTCCCCCGGACCCTCGCGTAACGCTCCCCGACCTCT

GGGAGAGAGGAGCGGAAGCTGAGGGTGTGAAACGGCTGGCCCCGAACACACCTCGCGGCG
 -----+-----+-----+-----+-----+-----+ 120
 CCGTCTCTCCTCGCCTTCGACTCCACACTTTGCCGACCGGGGCTTGTGTGGAGCGCCGC

CTCCAGTGATTCTTGGTGTCCGACCTCAGCCCCAGTCAGTGCGGGTCCAGTTTCCAGGCT
 -----+-----+-----+-----+-----+-----+ 180
 GAGGTACTAAGGACCACAGGCTGGAGTCGGGGTCAGTCACGCCCAGGTCAAAGGTCCGA

CTCGCGGAAGGCCTGGCTGAGCACATGCGGCAGCCACGGTCGCCCTCCCTATTCTCTTA
 -----+-----+-----+-----+-----+-----+ 240
 GAGCGCCTTCCGGACCGACTCGTGACGCCGTCGGTGCCAGCGGGAGGGATAAGGAGAAT

GCCCCGAGGAGGGGGGTCCCAAGTTACATGGCCACGCAGATGGGGCCTCTCCCTCATTCT
 -----+-----+-----+-----+-----+-----+ 300
 CGGGCTCCTCCCCCAGGGTTCAATGTACCGGTGCGTCTACCCCGGAGAGGGAGTAAAGA

GAACCTTGTGGGGAGGGGAACCTTGAAGGGAGCGCCCCCAGAGCCATGGCTTAGGGCCT
 -----+-----+-----+-----+-----+-----+ 360
 CTTGGAACACCCCTCCCTTGGAACTTCCCTCGCGGGGGTCTCGGTACCGAATCCCGGA

CCCCACCCCTCTGGAGCTCCAGTCTGCAAGAGTCAGGAGCCGAAATATCGCTGACTGTG
 -----+-----+-----+-----+-----+-----+ 420
 GGGGGTGGGGAGACCTCGAGGTGAGACGTTCTCAGTCCTCGGCTTTATAGCGACTGACAC

GGTGACGACTCTTGCGCGCACACACATACAAGCGGGCACGACGCGTTCGGTCTCTATTA
 -----+-----+-----+-----+-----+-----+ 480
 CCACTGCTGAGAACGCGCGTGTGTGTGTATGTTCCCGGTGCTGCGCAAGCCAGGATAAT

AAAGGCACGCAAGGGTGC GGCTGCACGCGGTGACACGGACCCCTCTAACGTTTCCAACT
 -----+-----+-----+-----+-----+-----+ 540
 TTTCCGTGCGTTCCACGCGGACGTGCGCCACTGTGCCTGGGGAGATTGCAAAGGTTTGA

GAGCTCCCTGCAGGTCCCCGACAGCACAGGCCCTGTCCCAGGACCCCTCCAGGCACGCG
 -----+-----+-----+-----+-----+-----+ 600
 CTCGAGGGACGTCCAGGGGCTGTGCTGTCGGGGACAGGGTCTTGGGGAGGTCCGTGCGC

CTCACACGCACACGCGCGCTCCCCGGCTCACGCGCGTCCGACACACACGCTCACGCGAA
 -----+-----+-----+-----+-----+-----+ 660
 GAGTGTGCGTGTGCGCGGAGGGGCCGAGTGC GCGGAGGCTGTGTGTGCGAGTGC GCTT

CGCAGGCGCACGCTCTGGCGCGGGAGGCGCCCTTCGCCTCCGTGTTGGGAAGCGGGGGC
 -----+-----+-----+-----+-----+-----+ 720
 GCGTCCGCGTGCGAGACCGCGCCCTCCGCGGGGAAGCGGAGGCACAACCTTCGCCCCCG

GGCGGGAGGGGCAGGAGACGTTGGCCCCGCTCGCGTTTCTGCAGCTGCTGCAGTCGCCGC
 -----+-----+-----+-----+-----+-----+ 780
 CCGCCCTCCCGTCTCTGCAACCGGGGCGAGCGCAAAGACGTCGACGACGTCAGCGGCG

AGCGTCCGGACCGGAACCAGCGCGCTCCGCGGAGCCGCGCCGCGCGCGGGGCCCTTT
 -----+-----+-----+-----+-----+-----+ 840
 TCGCAGGCCTGGCCTTGGTCCGCGCAGGCGCCTCGGCGGCGGCGGCGGCGGCGGCGGAAA

CCAAGCCGGGCGCTCGGAGCTGTGCCCCGGCCCCGCTTCAGCACCGCGGACAGCTCCGGCC
 -----+-----+-----+-----+-----+-----+ 900
 GGTTCGGCCCCGAGCCTCGACACGGGCGGGGCGAAGTCGTGGCGCCTGTGAGGCGCGG

FIGURE 1 - PAGE 2

```

CGGTGGGGCTGAGCCGAGCCCCGCGCACGCTTCAGCCCCCTTCCCTCGGCCGACGTCCC
-----+-----+-----+-----+-----+-----+ 960
CGCACCCCGACTCGGCTCGGGGCGCGTGCGAAGTCGGGGGAAGGGAGCCGGCTGCAGGG

GGGACCGCCGCTCCGGGGGAGACGTGGCGTCCGCGAGCCGCGGGGCCGGGCGAGCGCAGG
-----+-----+-----+-----+-----+-----+ 1020
CCCTGGCGGCGAGGCCCTCTGCACCGCAGGCGTCGGGCGCCCCGGCCCCGCTCGCGTCC

ACGCCCCGGAAGCCCCGCGGGGGATGCGCCGAGGGCCCGGTTTCGCGCCGCGCAGAGCCA
-----+-----+-----+-----+-----+-----+ 1080
TGCCGGGCCTTCGGGGCGCCCCCTACGCGGCTCCCGGGCGCAAGCGGGCGCGTCTCGGT

      .|-----signal-peptide-----
      M S T M R L L T L A L L F S -4
GGCCCGCGGCCCGAGCCCATGAGCACCATGCGCCTGCTGACGCTCGCCCTGCTGTTCTCC
-----+-----+-----+-----+-----+-----+ 1140
CCGGGCGCGGGGCTCGGGTACTCGTGGTACGCGGACGACTGCGAGCGGGACGACAAGAGG

-----|
C S V A R A A C D P K I V N I G A V L S 16
TGCTCCGTGCGCCGTGCCGCGTGCGACCCCAAGATCGTCAACATTGGCGCGGTGCTGAGC
-----+-----+-----+-----+-----+-----+ 1200
ACGAGGCAGCGGGCACGGCGCACGCTGGGGTTCTAGCAGTTGTAACCGCGCCACGACTCG

T R K H E Q M F R E A V N Q A N K R H G 36
ACGCGGAAGCACGAGCAGATGTTCCGCGAGGCCGTGAACCAGGCCAACAGCGGCACGGC
-----+-----+-----+-----+-----+-----+ 1260
TGCGCCTTCGTGCTCGTCTACAAGGCGCTCCGGCACTTGGTCCGGTTGTTTCGCGGTGCCG

S W K I Q L N A T S V T H K P N A I Q M 56
TCCTGGAAGATTTCAGCTCAATGCCACCTCCGTACGCACAAGCCCCAACGCCATCCAGATG
-----+-----+-----+-----+-----+-----+ 1320
AGGACCTTCTAAGTCGAGTTACGGTGGAGGCAGTGCCTGTTTCGGGTTGCGGTAGGTCTAC

A L S V C E D L I S S Q V Y A I L V S H 76
GCTCTGTGCGGTGTGCGAGGACCTCATCTCCAGCCAGGTCTACGCCATCCTAGTTAGCCAT
-----+-----+-----+-----+-----+-----+ 1380
CGAGACAGCCACACGCTCCTGGAGTAGAGGTTCGGTCCAGATGCGGTAGGATCAATCGGTA

P P T P N D H F T P T P V S Y T A G F Y 96
CCACCTACCCCAACGACCACTTCACTCCACCCCTGTCTCCTACACAGCCGGCTTCTAC
-----+-----+-----+-----+-----+-----+ 1440
GGTGGATGGGGGTTGCTGGTGAAGTGAGGGTGGGGACAGAGGATGTGTCGGCCGAAGATG

R I P V L G L T T R M S I Y S D K S I H 116
CGCATACCCGTGCTGGGGCTGACCACCCGCATGTCCATCTACTCGGACAAGAGCATCCAC
-----+-----+-----+-----+-----+-----+ 1500
GCGTATGGGCACGACCCCGACTGGTGGGCGTACAGGTAGATGAGCCTGTTCTCGTAGGTG

L S F L R T V P P Y S H Q S S V W F E M 136
CTGAGCTTCCTGCGCACCGTGCCGCCCTACTCCACCAAGTCCAGCGTGTGGTTTGAGATG
-----+-----+-----+-----+-----+-----+ 1560
GACTCGAAGGACGCGTGGCAGGCGGGGATGAGGGTGGTCAGGTCGCACACCAAACTCTAC

M R V Y S W N H I I L L V S D D H E G R 156
ATGCGTGTCTACAGCTGGAACCATCATCTGCTGGTCAGCGACGACCAGAGGGCCGG
-----+-----+-----+-----+-----+-----+ 1620
TACGCACAGATGTCGACCTTGGTGTAGTAGGACGACCAAGTCGCTGCTGGTGTCTCCGGCC

```

FIGURE 1 - PAGE 3

A A Q K R L E T L L E E R E S K A E K V 176
 GCGGCTCAGAAACGCCTGGAGACGCTGCTGGAGGAGCGTGAGTCCAAGGCAGAGAAGGTG
 -----+-----+-----+-----+-----+-----+1680
 CGCCGAGTCTTTGCGGACCTCTGCGACGACCTCCTCGCACTCAGGTTCCGTCTCTTCCAC

 L Q F D P G T K N V T A L L M E A K E L 196
 CTGCAGTTTGACCCAGGGACCAAGAACGTGACGGCCCTGCTGATGGAGGCGAAAGAGCTG
 -----+-----+-----+-----+-----+-----+1740
 GACGTCAAACCTGGGTCCCTGGTTCTTGCACTGCCGGGACGACTACCTCCGCTTTCTCGAC

 E A R V I I L S A S E D D A A T V Y R A 216
 GAGGCCCCGGGTCAATCATCCTTTCTGCCAGCGAGGACGATGCTGCCACTGTATACCGCGCA
 -----+-----+-----+-----+-----+-----+1800
 CTCCGGGCCCCAGTAGTAGGAAAGACGGTCGCTCCTGCTACGACGGTGACATATGGCGCGT

 A A M L N M T G S G Y V W L V G E R E I 236
 GCCGCGATGCTGAACATGACGGGCTCCGGGTACGTGTGGCTGGTCGGCGAGCGCGAGATC
 -----+-----+-----+-----+-----+-----+1860
 CGGCGCTACGACTTGTACTGCCCGAGGCCCCATGCACACCGACCGAGCCGCTCGCGCTCTAG

 S G N A L R Y A P D G I L G L Q L I N G 256
 TCGGGGAACGCCCTGCGCTACGCCCCAGACGGCATCCTCGGGCTGCAGCTCATCAACGGC
 -----+-----+-----+-----+-----+-----+1920
 AGCCCTTGCGGGACGCGATGCGGGGTCTGCCGTAGGAGCCCCGACGTCGAGTAGTTGCCG

 K N E S A H I S D A V G V V A Q A V H E 276
 AAGAACGAGTCGGCCACATCAGCGACGCCGTGGGCGTGGTGGCCAGGCCGTGCACGAG
 -----+-----+-----+-----+-----+-----+1980
 TTCTTGCTCAGCCGGGTGTAGTCGCTGCGGCACCCGACCCACCGGTCCGGCACGTGCTC

 L L E K E N I T D P P R G C V G N T N I 296
 CTCCTCGAGAAGGAGAACATCACCGACCCGCGCGGGGCTGCGTGGGCAACACCAACATC
 -----+-----+-----+-----+-----+-----+2040
 GAGGAGCTCTTCTCTGTAGTGGCTGGGCGGCGCCCCGACGCACCCGTTGTGGTTGTAG

 W K T G P L F K R V L M S S K Y A D G V 316
 TGGAAGACCGGGCCGCTCTTCAAGAGAGTGCTGATGTCTTCCAAGTATGCGGATGGGGTG
 -----+-----+-----+-----+-----+-----+2100
 ACCTTCTGGCCCGGCGAGAAGTTCTCTCAGCACTACAGAAGGTTTCATACGCCTACCCAC

 T G R V E F N E D G D R K F A N . Y . S . I M 336
 ACTGGTCGCGTGGAGTTCAATGAGGATGGGGACCGGAAGTTCGCCAACTACAGCATCATG
 -----+-----+-----+-----+-----+-----+2160
 TGACCAGCGCACCTCAAGTTACTCCTACCCCTGGCCTTCAAGCGGTTGATGTCGTAGTAC

 N L Q N R K L V Q V G I Y N G T H V I P 356
 AACCTGCAGAACCGCAAGCTGGTGCAAGTGGGCATCTACAATGGCACCCACGTCATCCCT
 -----+-----+-----+-----+-----+-----+2220
 TTGGACGTCTTGGCGTTCCGACCAGTTACCCGTTAGATGTTACCGTGGGTGCAGTAGGGA

 N D R K I I W P G G E T E K P R G Y Q M 376
 AATGACAGGAAGATCATCTGGCCAGGCGGAGACAGAGAAGCCTCGAGGGTACCAGATG
 -----+-----+-----+-----+-----+-----+2280
 TTACTGTCTTCTAGTAGACCGGTCCGCCTCTCTGTCTCTTCGGAGCTCCCATGGTCTAC

 S T R L K I V T I H Q E P F V Y V K P T 396
 TCCACCAGACTGAAGATTGTGACGATCCACCAGGAGCCCTTCGTGTACGTCAAGCCCACG
 -----+-----+-----+-----+-----+-----+2340
 AGGTGGTCTGACTTCTAACACTGCTAGGTGGTCCTCGGGAAGCACATGCAGTTCGGGTGC

FIGURE 1 - PAGE 4

```

L S D G T C K E E F T V N G D P V K K V 416
CTGAGTGATGGGACATGCAAGGAGGAGTTCACAGTCAACGGCGACCCAGTCAAGAAGGTG
-----+-----+-----+-----+-----+-----+-----+-----+-----+2400
GACTCACTACCCTGTACGTTCTCCTCAAGTGTGAGTTGCCGCTGGGTGAGTTCTTCCAC

I C T G P N D T S P G S P R H T V P Q C 436
ATCTGCACCGGGCCCAACGACACGTGCGCGGGCAGCCCCCGCCACACGGTGCCTCAGTGT
-----+-----+-----+-----+-----+-----+-----+-----+-----+2460
TAGACGTGGCCCCGGGTGCTGTGTCAGCGGCCCGTCCGGGGCGGTGTGCCACGGAGTCACA

C Y G F C I D L L I K L A R T M N F T Y 456
TGCTACGGCTTTTGCATCGACCTGCTCATCAAGCTGGCAGGACCATGAACCTCACCTAC
-----+-----+-----+-----+-----+-----+-----+-----+-----+2520
ACGATGCCGAAAACGTAGCTGGACGAGTAGTTCGACCGTGCCTGGTACTTGAAGTGGATG

E V H L V A D G K F G T Q E R V N N S N 476
GAGGTGCACCTGGTGGCAGATGGCAAGTTCGGCACACAGGAGCGGGTGAACAACAGCAAC
-----+-----+-----+-----+-----+-----+-----+-----+-----+2580
CTCCACGTGGACCACCGTCTACCGTTCAAGCCGTGTGTCCTCGCCCACTTGTGTGCTTG

K K E W N G M M G E L L S G Q A D M I V 496
AAGAAGGAGTGAATGGGATGATGGCGGAGTCTGCTCAGCGGGCAGGCAGACATGATCGTG
-----+-----+-----+-----+-----+-----+-----+-----+-----+2640
TTCTTCCTCACCTTACCCTACTACCGCTCGACGAGTCGCCCGTCCGTCTGTACTAGCAC

A P L T I N N E R A Q Y I E F S K P F K 516
GCGCCGCTAACCATAAACACGAGCGCGCAGTACATCGAGTTTCCAAGCCCTTCAAG
-----+-----+-----+-----+-----+-----+-----+-----+-----+2700
CGCGGCGATTGGTATTTGTTGCTCGCGCGCTCATGTAGCTCAAAGGTTTCGGGAAGTTC

Y Q G L T I L V K K E I P R S T L D S F 536
TACCAGGGCCTGACTATTCTGGTCAAGAAGGAGATTCCCCCGGAGCACGCTGGACTCGTTC
-----+-----+-----+-----+-----+-----+-----+-----+-----+2760
ATGGTCCCGGACTGATAAGACCAAGTCTTCTCTAAGGGGCCTCGTGCGACCTGAGCAAG

M Q P F Q S T L W L L V G L S V H V V A 556
ATGCAGCCGTTCCAGAGCACACTGTGGCTGCTGGTGGGGCTGTGCGGTGCACGTGGTGGCC
-----+-----+-----+-----+-----+-----+-----+-----+-----+2820
TACGTCCGCAAGGTCTCGTGTGACACCGACGACCAACCCCGACAGCCACGTGCACCACCGG

V M L Y L L D R F S P F G R F K V N S E 576
GTGATGCTGTACCTGCTGGACCGCTTCAAGCCCTTCGGCCGGTTCAAGGTGAACAGCGAG
-----+-----+-----+-----+-----+-----+-----+-----+-----+2880
CACTACGACATGGACGACCTGGCGAAGTCGGGGAAGCCGGCCAAGTCCACTTGTGCTC

E E E E D A L T L S S A M W F S W G V L 596
GAGGAGGAGGAGGACGCACTGACCTGTCTCGGCCATGTGGTTCTCTGGGGCGTCTTG
-----+-----+-----+-----+-----+-----+-----+-----+-----+2940
CTCCTCCTCCTCCTGCGTGAAGTGGGACAGGAGCCGGTACACCAAGAGGACCCCGCAGGAC

L N S G I G E G A P R S F S A R I L G M 616
CTCAACTCCGGCATCGGGGAAGGCGCCCCCAGAAGCTTCTCAGCGCGCATCTGGGCATG
-----+-----+-----+-----+-----+-----+-----+-----+-----+3000
GAGTTGAGGCCGTAGCCCCCTTCGCGGGGGTCTTCAAGAGTCCGCGGTAGGACCCGTAC

```

FIGURE 1 - PAGE 5

```

-----TM-3-----|
V W A G F A M I I V A S Y T A N L A A F 636
GTGTGGGCCGGCTTTGCCATGATCATCGTGGCCTCCTACACGCCAACCTGGCGGCCTTC
-----+-----+3060
CACACCCGGCCGAAACGGTACTAGTAGCACCAGGAGTGTGGCGGTTGGACCGCCGGAAG

L V L D R P E E R I T G I N D P R L R N 656
CTGGTGCTGGACCGGCCGAGGAGCGCATCACGGGCATCAACGACCCTCGGCTGAGGAAC
-----+-----+3120
GACCACGACCTGGCCGGCCTCCTCGCGTAGTGCCCGTAGTTGCTGGGAGCCGACTCCTTG

P S D K F I Y A T V K Q S S V D I Y F R 676
CCCTCGGACAAGTTTATCTACGCCACGGTGAAGCAGAGCTCCGTGGATATCTACTTCCGG
-----+-----+3180
GGGAGCCTGTTCAAATAGATGCGGTGCCACTTCGTCTCGAGGCACCTATAGATGAAGGCC

R Q V E L S T M Y R H M E K H N Y E S A 696
CGCCAGGTGGAGCTGAGCACCATGTACCGGCATATGGAGAAGCACAACCTACGAGAGTGCG
-----+-----+3240
GCGGTCCACCTCGACTCGTGGTACATGGCCGTATACCTCTTCGTGTTGATGCTCTCACGC

A E A I Q A V R D N K L H A F I W D S A 716
GCGGAGGCCATCCAGGCCGTGAGAGACAACAAGCTGCATGCCTTCATCTGGGACTCGGCG
-----+-----+3300
CGCCTCCGGTAGGTCCGGCACTCTCTGTTGTTTCGACGTACGGAAGTAGACCCTGAGCCGC

V L E F E A S Q K C D L V T T G E L F F 736
GTGCTGGAGTTCGAGGCCTCGCAGAAGTGCGACCTGGTGACGACTGGAGAGCTGTTTTTC
-----+-----+3360
CACGACCTCAAGCTCCGGAGCGTCTTCACGCTGGACCACTGCTGACCTCTCGACAAAAAG

R S G F G I G M R K D S P W K Q N V S L 756
CGCTCGGGCTTCGGCATAGGCATGCGCAAAGACAGCCCTGGAAGCAGAACGTCTCCCTG
-----+-----+3420
GCGAGCCCGAAGCCGTATCCGTACGCGTTTCTGTGCGGGACCTTCGTCTTGACAGGGGAC

S I L K S H E N G F M E D L D K T W V R 776
TCCATCCTCAAGTCCCACGAGAATGGCTTCATGGAAGACCTGGACAAGACGTGGGTTCGG
-----+-----+3480
AGGTAGGAGTTCAGGGTGCTCTTACCGAAGTACCTTCTGGACCTGTTCTGCACCCAAGCC

Y Q E C D S R S N A P A T L T F E N M A 796
TATCAGGAATGTGACTCGCGCAGCAACGCCCTGCGACCCTTACTTTTGAGAACATGGCC
-----+-----+3540
ATAGTCCTTACACTGAGCGCGTCGTTGCGGGGACGCTGGGAATGAAAACCTCTGTACCGG

-----TM-4-----|
G V F M L V A G G I V A G I F L I F I E 816
GGGGTCTTCATGCTGGTAGCTGGGGGCATCGTGGCCGGGATCTTCCTGATTTTCATCGAG
-----+-----+3600
CCCCAGAAGTACGACCATCGACCCCCGTAGCACCGGCCCTAGAAGGACTAAAAGTAGCTC

I A Y K R H K D A R R K Q M Q L A F A A 836
ATTGCCTACAAGCGGCACAAGGATGCTCGCCGGAAGCAGATGCAGCTGGCCTTTGCGGCC
-----+-----+3660
TAACGGATGTTGCGCGTGTTCTACGAGCGGCCTTCGTCTACGTCGACCGGAAACGGCGG

V N V W R K N L Q Q Y H P T D I T G P L 856
GTTAACGTGTGGCGGAAGAACCTGCAGCAGTACCATCCCACTGATATCACGGGCCCCGCTC
-----+-----+3720
CAATTGCACACCGCCTTCTTGACGTCGTATGGTAGGGTGACTATAGTGCCCGGGCGAG

```

FIGURE 1 - PAGE 6

N L S D P S V S T V V 867
 AACCTCTCAGATCCCTCGGTACGACCCGTGGTGTGAGGCCCCCGGAGGCGCCACCTGCC
 -----+-----+-----+-----+-----+3780
 TTGGAGAGTCTAGGGAGCCAGTCTGTGGACCACTCCGGGGGCTCCGCGGGTGGACGG

 CAGTTAGCCCGGCAAGGACACTGATGGGTCTCTGCTCGGGAAGGCCTGAGGGAAGCC
 -----+-----+-----+-----+-----+3840
 GTCAATCGGGCCGGTTCCTGTGACTACCCAGGACGACGAGCCCTTCCGGACTCCCTTCGG

 CACCCGCCCCAGAGACTGCCACCCCTGGGCCTCCCGTCCGTCCGCCCCGCCACCCGCTG
 -----+-----+-----+-----+-----+3900
 GTGGGCGGGTCTCTGACGGGTGGGACCCGGAGGGCAGGCAGGCGGGCGGGTGGGGCGAC

 CCTGGCGGGCAGCCCTGCTGGACCAAGGTGCGGACCGAGCGGCTGAGGACGGGGCAGA
 -----+-----+-----+-----+-----+3960
 GGACCGCCCGTCCGGGACGACCTGGTTCCACGCCTGGCCTCGCCGACTCCTGCCCCGTCT

 GCTGAGTCGGCTGGGCAGGGCGCAGGGCGCTCCGGCAGAGGCAGGGCCCTGGGGTCTCTG
 -----+-----+-----+-----+-----+4020
 CGACTCAGCCGACCCGTCCCGCGTCCCGCGAGGCGTCTCCGTCCCGGGACCCAGAGAC

 AGCAGTGGGGAGCGGGGGCTAACTGGCCCCAGGCGAAGGGGCTTGGAGCAGAGACGGCAG
 -----+-----+-----+-----+-----+4080
 TCGTACCCCTCGCCCCGATTGACCGGGGTCCGCTTCCCCGAACCTCGTCTCTGCCGTC

 CCCCATCCTTCCCGCAGCACCAGCCTGAGCCACAGTGGGGCCCATGGCCCCAGCTGGCTG
 -----+-----+-----+-----+-----+4140
 GGGGTAGGAAGGGCGTCTGGTCCGACTCGGTGTACCCCGGTACCGGGGTCCGACCGAC

 GGTGCCCCCTCCTCGGGCGCCTGCGCTCCTCTGCAGCCTGAGCTCCACCCTCCCTCTTC
 -----+-----+-----+-----+-----+4200
 CCAGCGGGGAGGAGCCCGCGGACGCGAGGAGACGTCGGAAGTGGGAGGGGAGAG

 TTGCGGCACCGCCACCCACACCCCGTCTGCCCTTGACCCACACGCGGGGGTGGCCC
 -----+-----+-----+-----+-----+4260
 AACGCCGTGGCGGGTGGGTGTGGGGCAGACGGGAAGTGGGGTGTGCGGCCCGACCGGG

 TGCCCTCCCCACGGCCGTCCCTGACTTCCAGCTGGCAGCGCCTCCCGCCGCTCGGGC
 -----+-----+-----+-----+-----+4320
 ACGGGAGGGGGTGGCGGCAGGACTGAAGGTGACCGTCCGCGAGGGCGGCGGAGCCCG

 CGCCTCCTCCAGACTCGAGAGGGCTGAGCCCTCCTCTCCTCGTCCGGCTGACAGCCAG
 -----+-----+-----+-----+-----+4380
 GCGGAGGAGGTCTGAGCTCTCCGACTCGGGGAGGAGAGGAGCAGGCCGGACGTCCGGTC

 AACGGGCCTCCCCGGGGGTCCCGGACGCTGGCTCGGGACTGTCTTCAACCTGCCCTGC
 -----+-----+-----+-----+-----+4440
 TTGCCCCGAGGGGCCCCAGGGGCTCGGACCGAGCCCTGACAGAAGTTGGGACGGGACG

 ACCTTGGGCACGGGAGAGCGCCACCCGCCCCGCCCCCGCCCTCGCTCCGGGTGCGTGACCG
 -----+-----+-----+-----+-----+4500
 TGGAACCCGTGCCCTCTCGCGGTGGGCGGGCGGGGGCGGGAGCGAGGCCACGCACTGGC

 GCCCGCCACCTTGTACAGAACCAGCACTCCCAGGGCCCGAGCGCTGCCCTCCCCGTGCG
 -----+-----+-----+-----+-----+4560
 CGGGCGGTGGAACATGTCTTGGTCTGAGGGTCCCGGGCTCGCGCACGGAAGGGGCACGC

 GCCCGTGCAGCGCGCTCTGCCCCCTCCGTCCCCAGGGTGCAGGCGCGACCGCCCAAC
 -----+-----+-----+-----+-----+4620
 CGGGCACGCGTCCGCGCAGACGGGGAGGCAGGGGTCCACGTCCGCGCGTGGCGGGTTG

 Eco RI
 CCCACCTCCCGGTGTATGCAGTGGTGATGCCGGAATTC
 -----+-----+-----+-----+-----+4659
 GGGGTGGAGGGCCACATACGTCACTACCGCCTTAAG

Figure 2

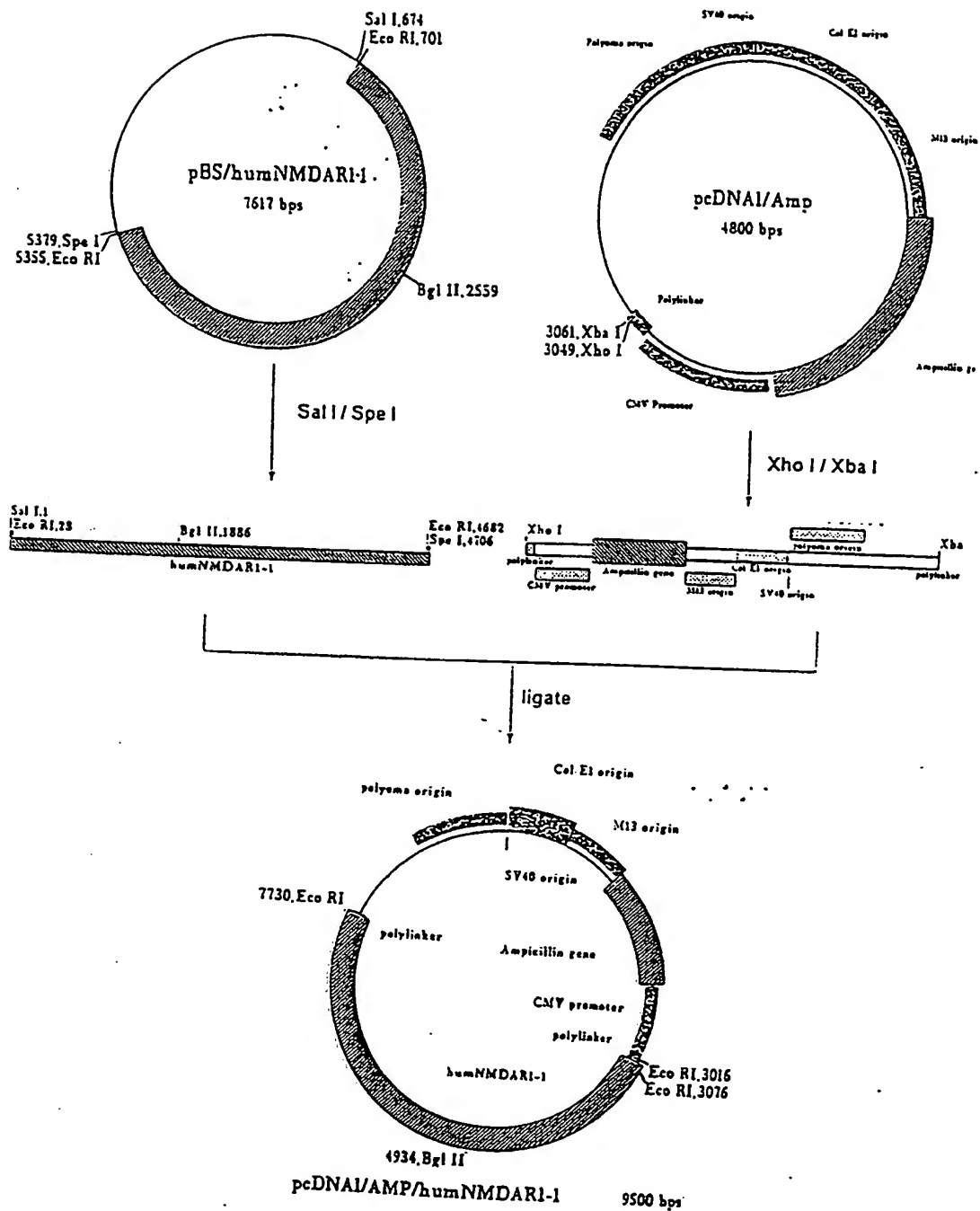


FIGURE 3A

```

3675 GAAGAACCTGCAG..... 3687 1
|||||
3675 GAAGAACCTGCAG..... 3687 2
|||||
3675 GAAGAACCTGCAGGATAGAAAGAGTGGTAGAGCAGAGCCTGACCCTAAAAAGAAAGCCAC +47 3A
|||||
3675 GAAGAACCTGCAGGATAGAAAGAGTGGTAGAGCAGAGCCTGACCCTAAAAAGAAAGCCAC +47 3C
|||||
..... 1
..... 2
ATTTAGGGCTATCACCTCCACCTGGCTTCCAGCTTCAAGAGGCGTAGGTCCCTCCAAAGA +107 3A
|||||
ATTTAGGGCTATCACCTCCACCTGGCTTCCAGCTTCAAGAGGCGTAGGTCCCTCCAAAGA +107 3C
|||||
..... 1
....AGCACCGGGGGTGGACGCGGCGCTTTGCAAAACCAAAAAGACACAGTGCTGCCGCG +56 2
|||||
CACGAGCACCGGGGGTGGACGCGGCGCTTTGCAAAACCAAAAAGACACAGTGCTGCCGCG +167 3A
|||||
CACGAGCACCGGGGGTGGACGCGGCGCTTTGCAAAACCAAAAAGACACAGTGCTGCCGCG +167 3C
|||||
..... 1
ACGCGCTATTGAGAGGGAGGAGGGCCAGCTGCAGCTGTGTTCCCGTCATAGGGAGAGCTG +116 2
|||||
ACGCGCTATTGAGAGGGAGGAGGGCCAGCTGCAGCTGTGTTCCCGTCATAGGGAGAGCTG +227 3A
|||||
ACGCGCTATTGAGAGGGAGGAGGGCCAGCTGCAGCTGTGTTCCCGTCATAGGGAGAGCTG +227 3C
|||||
*
END
..... 1
AGACTCCCCGCCCCCTCTCTGCCCCCTCCCCCGCAGACAGACAGACAGACGGATGGG +176 2
|||||
AGACTCCCCGCCCCCTCTCTGCCCCCTCCCCCGCAGACAGACAGACAGACGGATGGG +287 3A
|||||
AGACTCCCCGCCCCCTCTCTGCCCCCTCCCCCGCAGACAGACAGACAGACGGATGGG +287 3C
|||||
..... 1
ACAGCGGCCCCGCCCCACGCAGAGCCCCGGAGCACCACGGGGTCGGGGGAGGAGCACCCCC +236 2
|||||
ACAGCGGCCCCGCCCCACGCAGAGCCCCGGAGCACCACGGGGTCGGGGGAGGAGCACCCCC +347 3A
|||||
ACAGCGGCCCCGCCCCACGCAGAGCCCCGGAGCACCACGGGGTCGGGGGAGGAGCACCCCC +347 3C
|||||
..... 1
AGCCTCCCCCAGGCTGCGCCTGCCGCCCCGCGGTTGGCCGGCTGGCCGGTCCACCCCGT +296 2
|||||
AGCCTCCCCCAGGCTGCGCCTGCCGCCCCGCGGTTGGCCGGCTGGCCGGTCCACCCCGT +407 3A
|||||
AGCCTCCCCCAGGCTGCGCCTGCCGCCCCGCGGTTGGCCGGCTGGCCGGTCCACCCCGT +407 3C
|||||
..... 1
CCCCGCCCCGCGCGTGCCCCCAGCGTGGGGCTAACGGGCGCCTTGTCTGTGTATTTCTAT +356 2
|||||
CCCCGCCCCGCGCGTGCCCCCAGCGTGGGGCTAACGGGCGCCTTGTCTGTGTATTTCTAT +467 3A
|||||
CCCCGCCCCGCGCGTGCCCCCAGCGTGGGGCTAACGGGCGCCTTGTCTGTGTATTTCTAT +467 3C
|||||
3688 .....CAGTACCATCCCACT 3702 humNMDAR1-1
|||||
+357 TTTGCAGCAGTACCATCCCACT 4065 humNMDAR1-2
|||||
+468 TTTGCAGCAGTACCATCCCACT 4176 humNMDAR1-3A
|||||
+468 TTTGCAGCAGTACCATCCCACT 4176 humNMDAR1-3C
|||||

```

FIGURE 3B

TM4

803	AGGIVAGIFLIFIEIAYKRHKDARRKQMLAFAAVNVWRKNLQYHPTDITGPLNLS DPS	862	1
803	AGGIVAGIFLIFIEIAYKRHKDARRKQMLAFAAVNVWRKNLQ.....	845	2
803	AGGIVAGIFLIFIEIAYKRHKDARRKQMLAFAAVNVWRKNLQDRKSGRAEPDPKKKATF	862	3A
803	AGGIVAGIFLIFIEIAYKRHKDARRKQMLAFAAVNVWRKNLQDRKSGRAEPDPKKKATF	862	3C

VSTVVSTGGGRGALQNQKDTVLPRAIEREEGQLQLCSRHRRES	867	1
		883	2
RAITSTLASSFKRRRSSKDTSTGGGRGALQNQKDTVLPRAIEREEGQLQLCSRHRRES	920	3A	
RAITSTLASSFKRRRSSKDTSTGGGRGALQNQKDTVLPRAIEREEGQLQLCSRHTES	920	3C	

FIGURE 4

humNMDAR1-1/humNMDAR1-3C

462	A D G K F G T Q E R V N N S N K K E W	481
2534	TGGCAGATGGCAAGTTCGGCACACAGGAGCGGGTGAACAACAGCAACAAGAAGGAGTGGA	2593
2534	TGGCAGATGGCAAGTTCGGCACACAGAAGCGGGTGAACAACAGCAACAAGAAGGAGTGGA	2593
462	A D G K F G T Q K R V N N S N K K E W	481

humNMDAR1-3B

FIGURE 5A

```

3675 GAAGAACCTGCAG..... 3687 1
      |||||
3675 GAAGAACCTGCAGGATAGAAAGAGTGGTAGAGCAGAGCCTGACCCTAAAAAGAAAGCCAC +47 4
      .....
+48  ATTTAGGGCTATCACCTCCACCCTGGCTTCCAGCTTCAAGAGGCGTAGGTCCTCCAAAGA +107 4
      .....
3688 ....CAGTACCATCCCACT 3702 humNMDAR1-1
      |||||
+108 CACGCAGTACCATCCCACT 3813 humNMDAR1-4

```

FIGURE 5B

```

      TM4
803 AGGIVAGIFLIFIEIAYKRHKDARRKQMLAFAAVNVWRKNLQ..... 845 1
      |||||
803 AGGIVAGIFLIFIEIAYKRHKDARRKQMLAFAAVNVWRKNLQDRKSGRAEPDPKKKATF 862 4
      .....
846 .....QYHPTDITGPLNLSDPSTVV 867 humNMDAR1-1
      |||||
863 RAITSTLASSFKRRRSSKDTQYHPTDITGPLNLSDPSTVV 904 humNMDAR1-4

```


FIGURE 6A

```

1649 TGGAGGAGCGTGAGTCCAAGAGTAAAAAAGGAACTATGAAAACCTCGAC +35 5,6,7,8
      |||||||
1649 TGGAGGAGCGTGAGT..... 1663 1,2,3,4

+36 CAACTGTCCTATGACAACAAGCGCGGACCCAAGGCAGAGAAGGTGCTGCA 1748 humNMDAR1-5 to 1-8
      |||||||
1664 .....CCAAGGCAGAGAAGGTGCTGCA 1685 humNMDAR1-1 to 1-4

```

FIGURE 6B

```

160 KRLETLLLEERESKSKRNYENLDQLSYDNKRGPKAQVLQFDPGTKN 206 humNMDAR1-5 to 1-8
      |||||||
160 KRLETLLLEERESK.....AEKVLQFDPGTKN 185 humNMDAR1-1 to 1-4

```

Figure 7

[3H]MK-801 Binding humNMDA R1-1

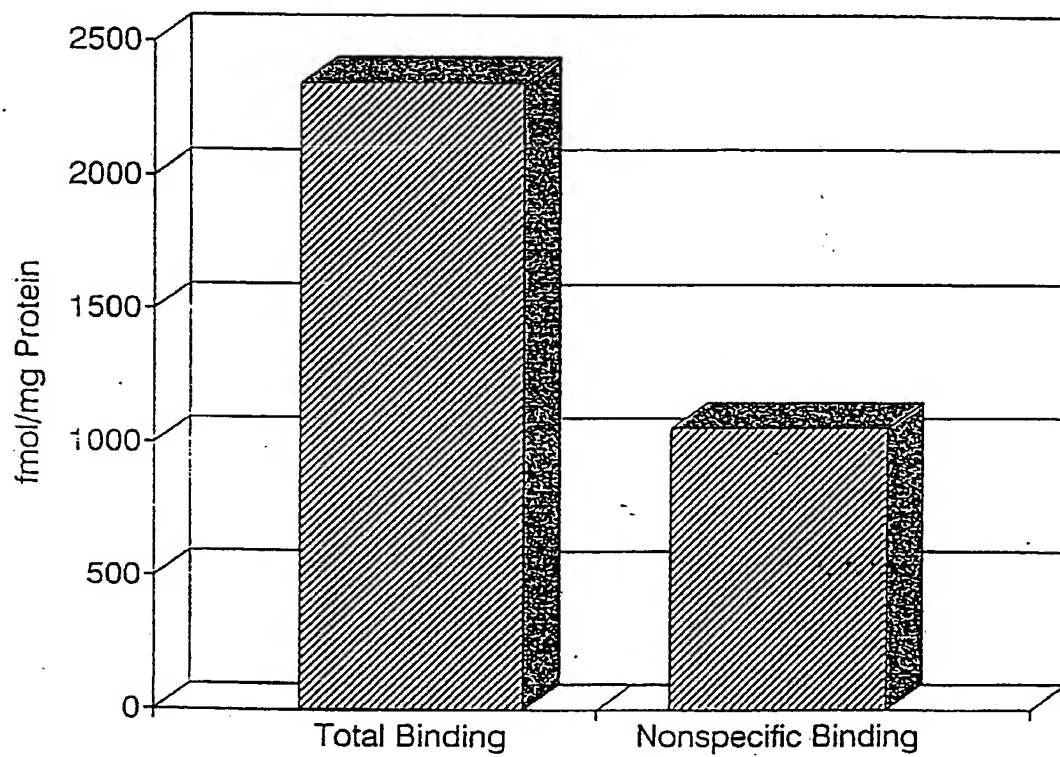
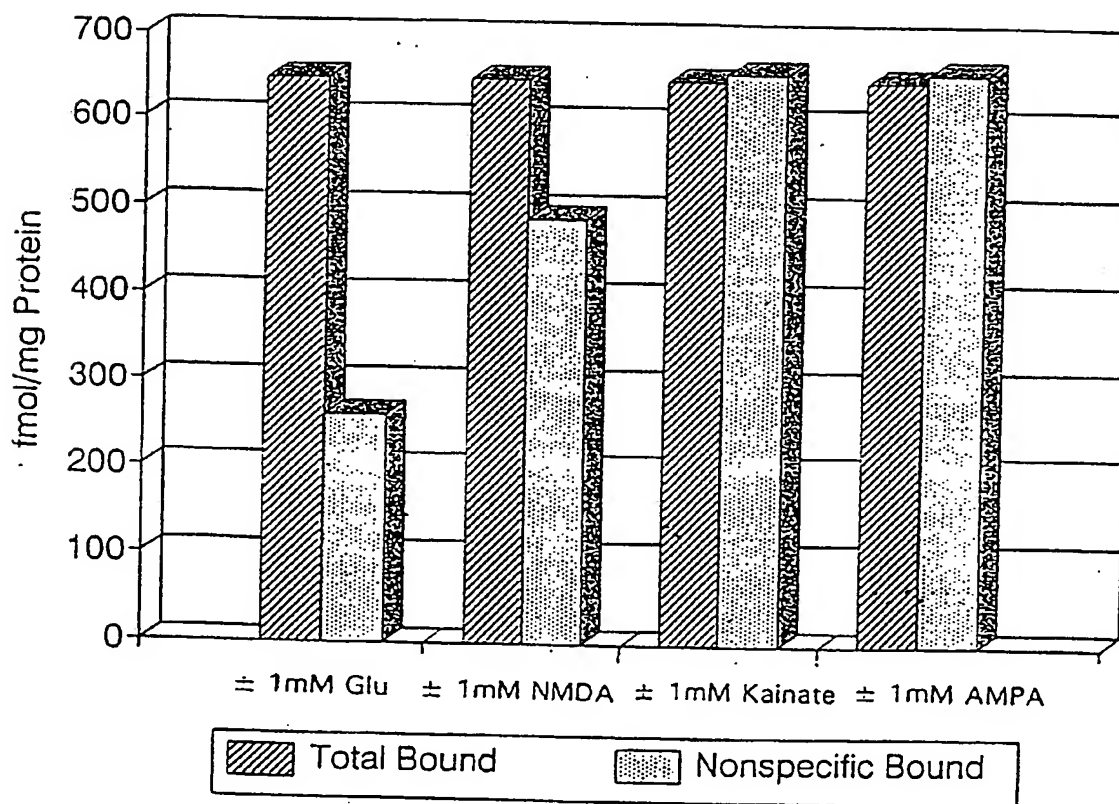


Figure 8

[3H]GLU Binding humNMDA R1-1





European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 93 30 9950

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
D,X	NATURE vol. 354 , 1991 pages 31 - 37 K. MORIYOSHI ET AL.; 'Molecular cloning and characterization of the rat NMDA receptor' *abstract; figures 2 and 3* ---	1,2,4-7, 12-15	C12N15/12 C07K15/06 C12N5/10 C12P21/00 G01N33/50 C12P21/08 C12Q1/68
D,X	FEBS LETT. vol. 300 , 1992 pages 39 - 45 M. YAMAZAKI ET AL.; 'Cloning, expression and modulation of a mouse NMDA receptor subunit' *abstract; introduction; figure 1* ---	1,2,4-7, 12-15	
A	WO-A-91 06648 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) *claims* ---	1	
A	J. PHARMACOL. EXPER. THER. vol. 260 , 1992 pages 1209 - 1213 I. OYE ET AL.; 'Effects of ketamine on sensory perception: evidence for a role of N-methyl-D-aspartate receptors' *abstract* ---	1	TECHNICAL FIELDS SEARCHED (Int.Cl.5) C07K
P,X	GENE vol. 131 , 1993 pages 293 - 298 R.L. FOLDES ET AL.; 'Cloning and sequence analysis of cDNAs encoding human hippocampus N-methyl-D-aspartate receptor subunits: evidence for alternative RNA splicing' *whole document* --- -/--	1-15	
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 11 May 1994	Examiner Yeats, S
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- A : member of the same patent family, corresponding document	

EPO FORM 150 (03.92) (P04C01)



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 93 30 9950

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
P,X	J. BIOL. CHEM. vol. 268 , 1993 pages 3728 - 3733 S.J. CARP ET AL.; 'Molecular cloning and chromosomal localization of the key subunit of the human N-methyl-D-aspartate receptor' *abstract; figure 1* ---	1-15	
E	WO-A-93 25679 (RHONE-POULENC RORER S.A.) *examples 5-7; SEQ ID NO:1; claims* -----	1-15	
			TECHNICAL FIELDS SEARCHED (Int.Cl.5)
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 11 May 1994	Examiner Yeats, S
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

EPO FORM 1503 (12/93) (P/4000)